



# Seed germination of bromeliad species from the *campo rupestre*: thermal time requirements and response under predicted climate-change scenarios<sup>☆</sup>

Alexandre Aparecido Duarte<sup>a</sup>, José Pires de Lemos Filho<sup>a</sup>, Andréa Rodrigues Marques<sup>b,\*</sup>

<sup>a</sup> Departamento de Botânica, ICB/UFMG, Av. Antônio Carlos, 6627, Pampulha, 31270110, Belo Horizonte, Minas Gerais, Brazil

<sup>b</sup> Departamento de Ciência e Tecnologia Ambiental, CEFET/MG, Av. Amazonas, 5253, Nova Suíça, 30480000, Belo Horizonte, Minas Gerais, Brazil

## ARTICLE INFO

### Article history:

Received 26 December 2016

Received in revised form 16 May 2017

Accepted 24 May 2017

Edited by Dr p Morellato

Available online 31 May 2017

### Keywords:

Base temperature

Bromeliad

*Campo rupestre*

Climate change

IPCC scenarios

Thermal time model

## ABSTRACT

The characterization of thermal thresholds, base ( $T_b$ ) and ceiling ( $T_c$ ) temperatures and thermal-times for germination ( $\theta_g$ ) has gained importance for predicting the persistence and distribution of species in the face of climate change. This study aimed to determine the thermal thresholds for germination of bromeliads from *campos rupestres* in order to verify if sympatric species have similar thermal requirements. We also examined whether inter-annual variations in climatic conditions drive differences on thermal requirements; and how the predicted climate change scenarios would influence the thermal time for germination. Seeds of *Vriesea friburgensis*, *V. bituminosa*, *V. pardalina*, *Tillandsia gardneri*, and *Racinaea aerisicola* were collected in 2000 and 2013 and sown to germinate in a temperature gradient (15–35 °C) under light and dark conditions. A thermal-time approach was then applied to the germination results, and the thermal-times needed to reach 50% germination ( $\theta_{50}$ ) were modeled according to the climatic conditions of both years, as well as two simulated scenarios climate change. The bromeliads exhibited differences in their thermal requirements for germination.  $T_b$ 's of from 6.2 to 10 °C and  $T_c$ 's of from 31.6 to 41.7 °C were identified, depending on the species. The extent and severity of the dry season during fruiting appeared to have determined differences in  $\theta_{50}$  values. All species, except *V. pardalina*, which have fruiting during the rainy season, presented higher  $\theta_{50}$  values in the year with a more intense drought (2013). Under the warming scenarios, a reduction in the time required to sum heat units to germinate was found, but a risk for persistence was identified for *R. aerisicola* in the *campos rupestres* due to its narrow seed germination window. The determination of thermal thresholds for germination can be useful to identify species vulnerability to climate change and to promote strategies for conservation of bromeliads species.

© 2017 Elsevier GmbH. All rights reserved.

## 1. Introduction

Regeneration niches and reproductive success are related to environmental temperatures, so that essentially all aspects of plant reproductive cycles are potentially sensitive to climate change (Menzel et al., 2006; Gordo and Sanz, 2009, 2010; Hoffmann et al., 2010; Bykova et al., 2012). The Intergovernmental Panel on Climate Change (IPCC) has predicted temperature increases of approximately 2–6 °C by 2090–2099 according to different greenhouse gas emission scenarios. Predictions have been grouped into four scenarios (A1, A2, B1, and B2) that consider alternative develop-

ment pathways, covering a wide range of demographic, economic and technological forces driving greenhouse gas emissions (IPCC, 2014). In face of climate change, plants may migrate to track their climatic niches (Meineri et al., 2013) or they may compensate by altering phenological events (e.g., earlier budding or flowering may lead to earlier seed set, or be compensated for by later seed development and maturation) (Post et al., 2008; Hoffmann et al., 2010). Attempts have been made to include phenology as a plastic response to climate change in models (e.g. Valladares et al., 2014). Theoretically, adaptive phenotypic plasticity should be greater in species regularly experiencing environmental heterogeneity and presumably having wider fundamental niches (Valladares et al., 2007; Valladares et al., 2014). Genotype and phenotype differences interact with environmental factors so that fitness may vary among populations as a function of both local adaptations and local environments (Molina-Montenegro et al., 2010; Nicotra et al., 2010;

<sup>☆</sup> This article is part of a special issue entitled Plant life in *campo rupestre*: new lessons from an ancient biodiversity hotspot published at the journal FLORA 238C.

\* Corresponding author.

E-mail address: [andreamg@gmail.com](mailto:andreamg@gmail.com) (A.R. Marques).

Banta et al., 2012; Gratani, 2014), although that type of information is still very scarce for Neotropical species.

Bromeliaceae comprises one of the most morphologically, physiologically, and ecologically distinct clade of Neotropical Angiosperms, with >3300 described species (Luther, 2008; Govaerts et al., 2012) distributed among eight subfamilies (Givnish et al., 2011). Bromeliads are one of the most important, diverse, and conspicuous elements of *campo rupestre* (CR) vegetation in Brazil (Versieux and Wendt, 2007; Versieux et al., 2010; Conceição and Pirani, 2016), but many species are threatened by pervasive anthropogenic impacts caused by overharvesting, mining, and habitat destruction (IUCN, 2001; Versieux and Wendt, 2007). CR is a heterogeneous, rocky, mountaintop (>900 m asl), grassland vegetation mostly occurring in the Espinhaço Range, in southeastern Brazil (Bitencourt et al., 2016; Fernandes, 2016). This species-rich vegetation is controlled by topography and pedo-environmental as well as micro-climatic conditions (Benites et al., 2007; Jacobi et al., 2007; Alves and Kolbek, 2010; Moreira et al., 2013; Schaefer et al., 2016; Silveira et al., 2016) generating remarkable habitat heterogeneity and, consequently, a great diversity (and combinations) of cues required for seed germination (Marques et al., 2014). Phenotypic plasticity can increase the ability of plants to survive water stress situations (Picotte et al., 2009), and the investigation of seed germination traits will be important to our full understanding of plant responses to the variable availability of water resources in CR. A study with twelve bromeliad species showed that the variation of germination responses to environmental conditions was not random with habitat-generalist plants showing broader germination niches as compared with habitat-specialist (Marques et al., 2014).

In spite of the importance of characterizing the thermal-time requirements of seed germination and understanding the regeneration niches of bromeliads to assess their extinction risks due to global warming (Müller et al., 2016), little attention has been given to thermal thresholds [i.e., the base temperatures ( $T_b$ ) and thermal-times required for germination ( $\theta_g$ )] in estimating the impacts of projected climate change scenarios. The germination responses of non-dormant seeds to temperature have been modeled using a thermal-time ( $\theta$ ) approach (e.g., García-Huidobro et al., 1982; Covell et al., 1986; Hardegree, 2006). In this model, seeds must accumulate thermal-time units (degree-days) for a percentage,  $g$ , of the population to germinate. When seeds are exposed to temperatures ( $T$ ) above their base germination temperature ( $T_b$  – which their germination rate is zero), but below their optimum temperature ( $T_o$  – which germination rates start to decrease; i.e., the sub-optimal temperature range), their germination rates increase linearly with temperature (García-Huidobro et al., 1982). Thus, in this temperature range, germination occurs at time  $t_g$  when the thermal-time accumulation has reached the critical value ( $\theta_g$ ) for a percentage  $g$  of the population. This response can be described as  $\theta_g = (T - T_b)t_g$ .

Given that the thermal thresholds for seed germination can vary according to environmental conditions during seed development (Daws et al., 2004; Chantre et al., 2009; Pérez-García, 2009; Porceddu et al., 2013; Sales et al., 2013; Baskin and Baskin, 2014), the present study sought to determine the thermal requirement for seed germination for bromeliads from CR collected during different climatic periods. Three questions were therefore raised: (1) Do sympatric bromeliads species have similar response patterns of thermal parameters?; (2) How do the inter-annual variations in climatic conditions drive differences on thermal requirements?; (3) How would germination time respond under predicted climate change scenarios?

We hypothesized that all sympatric bromeliads would: (1) probably show similar patterns of base ( $T_b$ ) and ceiling ( $T_c$ ) temperatures as well as thermal times; (2) that inter-annual differences in rainfall and temperature would promote differences in thermal

**Table 1**

Collection months, microhabitats, and the distributions of five bromeliad species in the Serra da Piedade Mountains, southeastern Brazil: xeric (x) and mesic (m); montane semi-deciduous forest (S), *campo rupestre* (CR), and altitudinal cloud forest (A).

Species	Collection months		Microhabitat <sup>1</sup>	Vegetation <sup>1</sup>
	2000	2013		
<i>Vriesea friburgensis</i>	Aug	Aug	m	S/CR
<i>Vriesea bituminosa</i>	Mar	Mar	x/m	A/CR
<i>Vriesea pardalina</i>	Apr	Apr	m	S/A
<i>Tillandsia gardneri</i>	Mar	Mar	x/m	S/CR
<i>Racinaea aerisicola</i>	Oct	Oct	m	A

<sup>1</sup> Data are from Marques et al. (2014).

requirements for germination, and; (3) that climate change would alter the number of days of environmental heat sum (HS) necessary to germinate 50% of the seeds in the population. Germination tests were carried out to test these hypotheses, and followed by probit analyses. The relationships between phenology and thermal requirements for seed germination were investigated, taking in account intra-annual changes in the drought index and the temperatures in two different years; the HS were calculated utilizing the average daily temperature of the first month with rainfall after seed dispersal for each species, as well as the projected temperatures under different climate change scenarios (+2 and +6 °C).

## 2. Material and methods

### 2.1. Study species

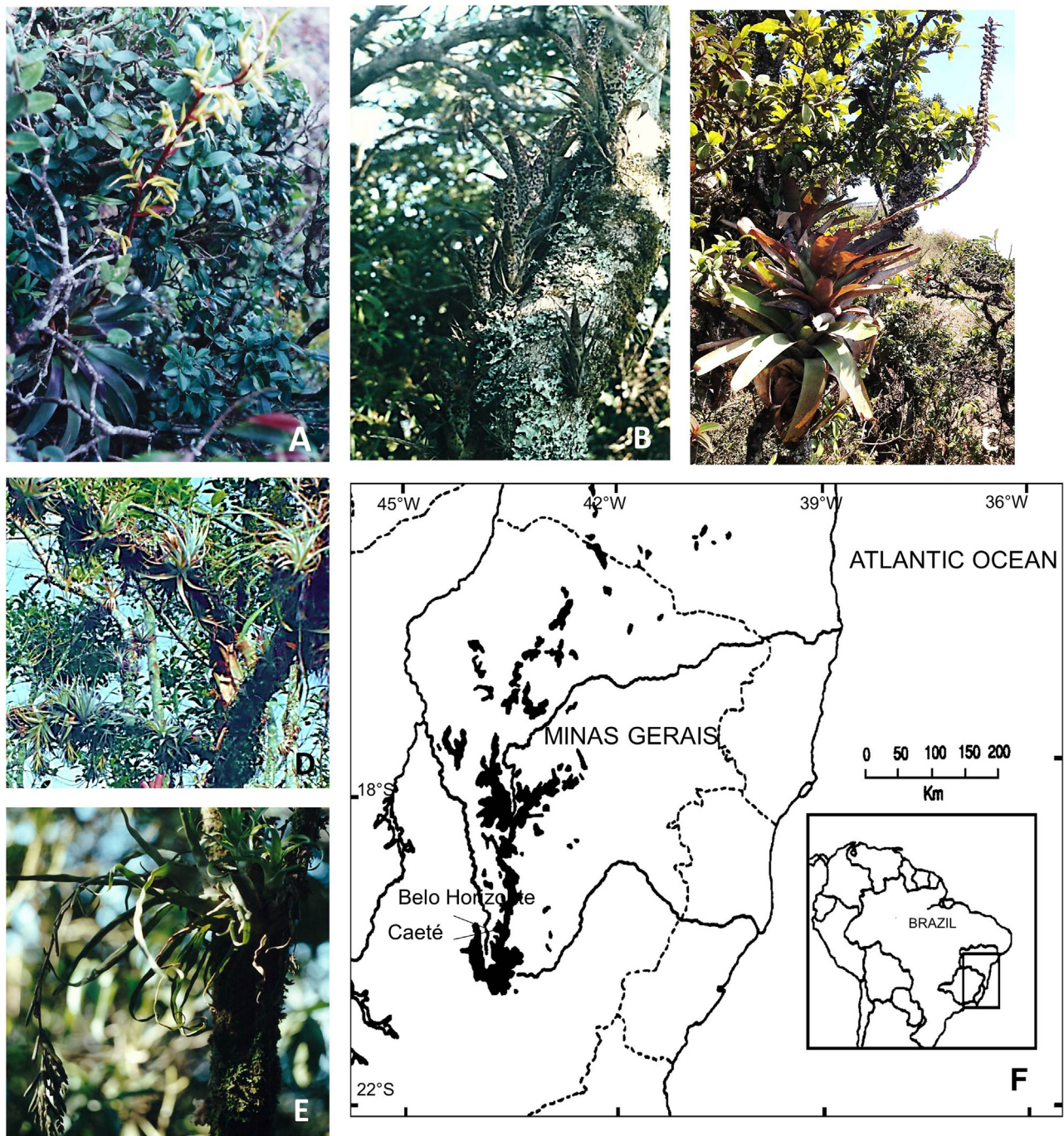
This study focused on five species of subfamily Tillandsioideae (Table 1): *Vriesea friburgensis* Mez (Fig. 1A), *V. bituminosa* Wawra (Fig. 1B), *V. pardalina* Mez (Fig. 1C), *Tillandsia gardneri* Lindl. (Fig. 1D) and *Racinaea aerisicola* (Mez) M. A. Spencer. & L. B. Sm. (Fig. 1E); which were localized at Serra da Piedade (Caeté) (19°48'–19°50'S, 43°39'–43°42'W), in the south portion of the Espinhaço Range, southeastern Brazil (Fig. 1F).

Seeds were collected in 2000 and 2013 during the peak seed production period of each species (Marques and Lemos-Filho, 2008; Fig. 2), and always from the same population (but from different individuals) (Table 1). To obtain representative seed samples, mature fruits were collected from at least 10 individuals of each species; 1500–2500 seeds were then mixed for the germination assays. Seeds were maintained in dark pots at room temperature (22 ± 2 °C) until use (approximately seven days).

### 2.2. Germination experiments

We evaluated germination traits by performing experiments at constant temperatures of 15, 20, 25, 30, and 35 ± 0.5 °C under both light (12-h photoperiod) and dark conditions. Four replicates of 25 seeds from the year 2000 and five replicates of 20 seeds from 2013 were sown onto filter paper moistened with distilled water and then held in germination boxes for 30 consecutive days under a photosynthetic photon flux density of 30 μmol m<sup>-2</sup> s<sup>-1</sup> (Marques et al., 2014). For the dark treatment, seed batches were placed in black germination boxes and germination was evaluated under a green safelight (490–560 nm) for 30 consecutive days. Seeds were considered germinated after their hypocotyl reached 0.5 mm in length (Marques et al., 2014). To ensure that no systematic effects occurred due to the positions of the experimental units within the incubators, the boxes were periodically randomly re-arranged. We calculated the final germination percentage (germinability;  $G\%$ ) and germination rate (GR) for each replicate (Ranal and Santana, 2006):

$$GR = 1/\bar{T}, \quad (1)$$



**Fig. 1.** *Vriesea friburgensis* in its terrestrial habit under a shrub, and a *Vriesea pardalina* epiphyte (B) in an altitudinal cloud forest (ACF) (A). *Vriesea bituminosa* epiphyte (C) on a shrub on a rocky outcrop. *Tillandsia gardneri* (D) and *Racinaea aerisicola* (E) epiphytes in the understory of a montane semi-deciduous forest and in an ACF respectively. Location of the study site, in the Serra da Piedade Mountains in Caeté, Minas Gerais State (F): the dark areas correspond to altitudes above 1000 m asl in the Espinhaço Mountain Range.

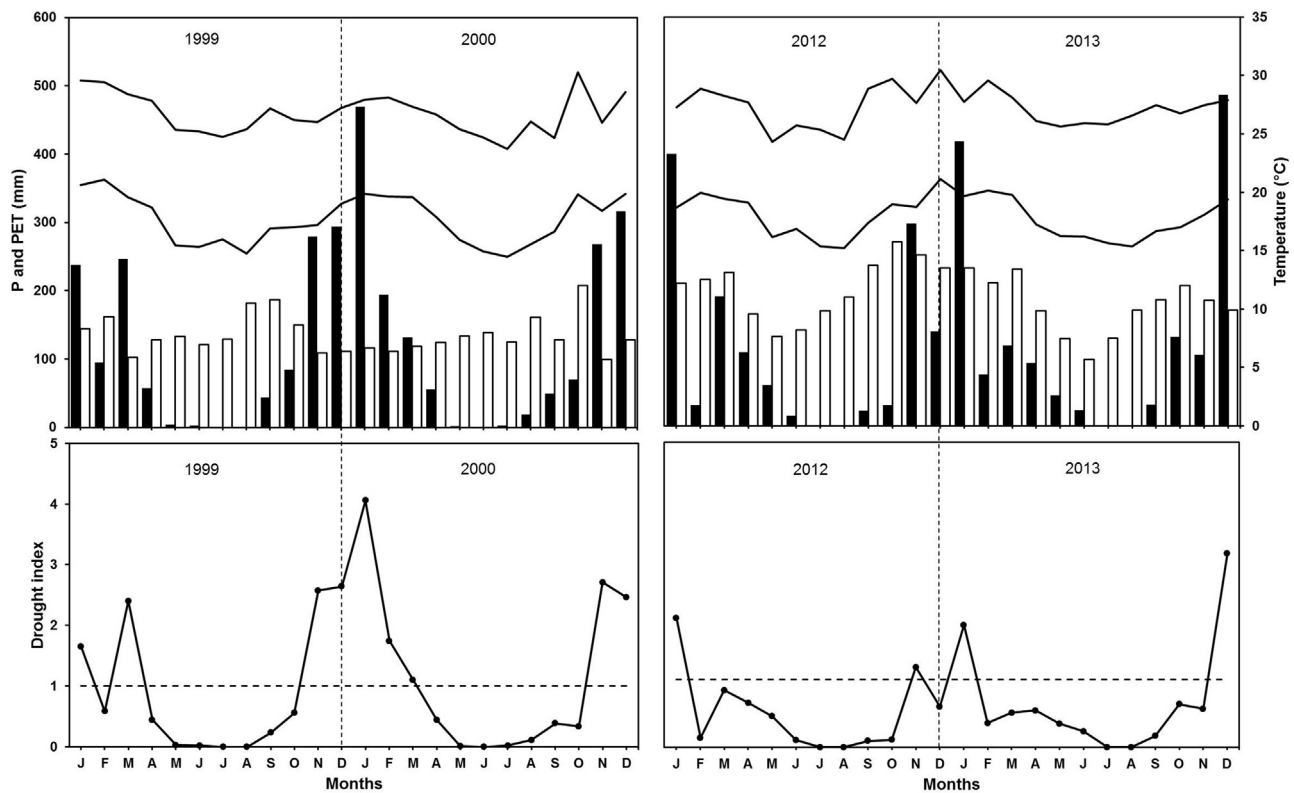
where  $\bar{T}$  the mean germination time ( $\bar{T} = \sum_{i=1}^k n_i t_i / \sum_{i=1}^k n_i$ );  $n_i$  is the number of seeds germinated in the time  $i$ ,  $t_i$  is the time from the start of the experiment to the  $i$ th observation, and  $k$  is the time of last germination. We considered the optimum temperature ( $T_o$ ) condition for seed germination to be the one with the highest GR.

### 3.1. Thermal-time model

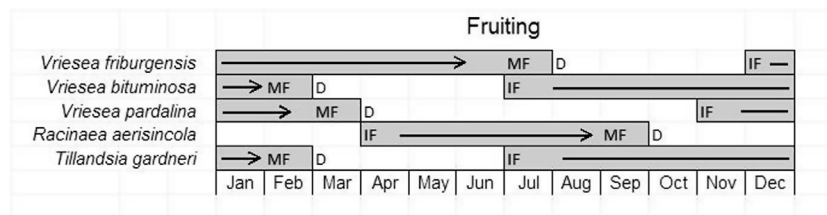
Despite discussions concerning how to best conduct experiments to calculate thermal times from germination percentage data

transformed to probit (see McNair et al., 2012; Hay et al., 2014), we opted for an experimental design with non-independent data. Bradford (1995) concluded that the germination data would be the same whether collected from dependent or independent samples, and that probit analysis is a valid approach.

Based on this assumption, germination percentages were transformed to probits (Finney, 1971). Linear regression was used to express probit( $g$ ) as a function of the log of thermal-time ( $\log \theta_g$ ) considering the base temperature ( $T_b$ ), below which there's no germination (Hardegree, 2006). Using the data from sub-optimal temperature range the base temperature ( $T_b$ ) was determined by



**Fig. 2.** Mean monthly minimum and maximum temperatures (solid line), precipitation (black bars), evapotranspiration (white bars), and drought index (ratio of precipitation to evapotranspiration) for 1999–2000 and 2012–2013. Drought index below one indicates water stress (dashed line). P = Precipitation, ET = EvapoTranspiration.



**Fig. 3.** Phenological patterns of fruit and seed maturation in *Vriesea friburgensis*, *V. bituminosa*, *V. pardalina*, *Tillandsia gardneri*, and *Racinaea aerisincola* in the Serra da Piedade Mountains, Minas Gerais State. IF = Immature Fruit, MF = Mature Fruit, D = Dispersal.

simulating using Eq. (3) (Covell et al., 1986). The simulate temperature that resulted in the highest  $r^2$  value of the probit equation was considered the ideal value of  $T_b$ .

$$\text{probit}(g) =$$

$$K + \{\log[(T - T_b)t_g]\}/\sigma \text{ then } \text{probit}(g) = K + \log\theta_g/\sigma, \quad (2)$$

where K is an intercept constant when the thermal time ( $\theta_g$ ) is zero, T is temperature,  $T_b$  is base temperature,  $t_g$  is timing for a fraction (g) (20–80%) of seed germinated. The  $\sigma$  is the standard deviation of the response to  $\theta_g$  (i.e. the reciprocal of the slope) and represents the sensitivity of the population to  $\theta_g$  (Covell et al., 1986). On a plot of probit(g) against  $\log \theta_g$ , the median thermal time required for seed germination of the population ( $g=50\%$ ;  $\theta_{50}$ ) corresponds to the thermal time when  $\text{probit}(g)=5$  (Finney, 1971).

Alternatively, for supra-optimal temperatures, the probit transformed percentages were  $\log T_c$  regressed, and different  $\theta$  were tested until attaining the best fit. The model was (Ellis et al., 1986):

$$\text{Probit}(g) =$$

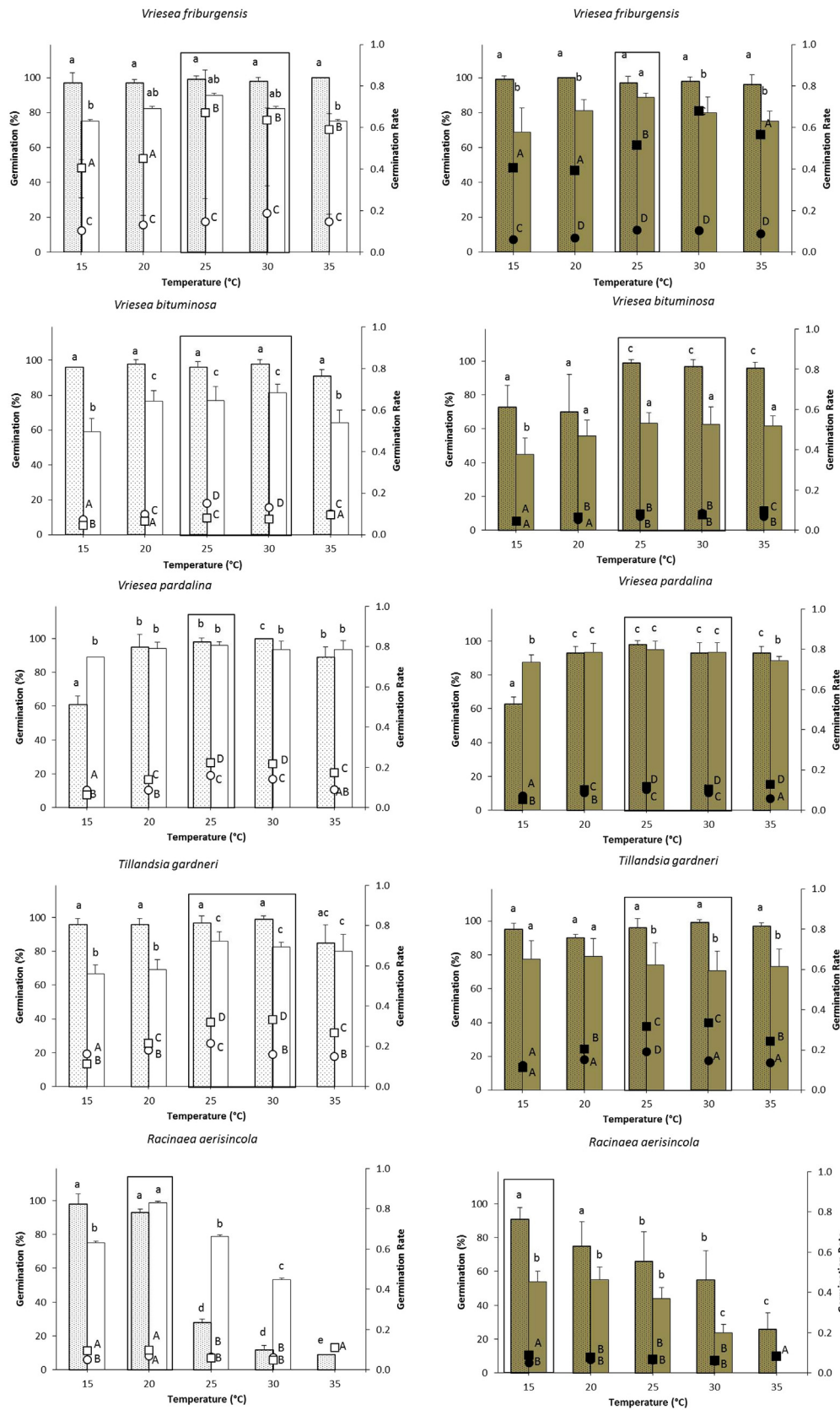
$$K + \{\log[T + (\theta/t_g)]\}/\sigma \text{ then } \text{probit}(g) = K + \log T_c/\sigma, \quad (3)$$

where K is an intercept constant of X axis, T is temperature,  $T_c$  is ceiling temperature,  $t_g$  is the time needed for a fraction (g) (20–80%) of the seed to germinate;  $\sigma$  is the standard deviation of the distribution of  $T_c$  in the seed population.

### 3.2. Climatic characterization of study site

The study site range in altitude from 1300 to 1700 m asl, with marked changes in vegetation with increasing altitudes (Marques et al., 2012). The climate at the study site is mesothermic (CwB according to the Köppen), with well-defined rainy summers and dry winters. The average temperature of the coldest and warmest month were 19.5 °C and 29.2 °C respectively, and average annual precipitation was above 1215 mm.

Meteorological data (precipitation, evapotranspiration potential, and temperature) from a conventional weather station maintained by the Instituto Nacional de Meteorologia (INMET) in the city of Belo Horizonte (approximately 40 Km from Serra da Piedade) (Fig. 3) were used to calculate the drought index ( $D_i$ ) (Picotte et al., 2009; Vicente-Serrano et al., 2010) and the environmental heat sum (HS) (Daws et al., 2004) or each month from 1999 to 2000 and from 2012 to 2013.



**Fig. 4.** Germination percentages (G% = bar) and germination rates (RG = symbol) (average  $\pm$  SE) of five species of Bromeliaceae from the Serra da Piedade Mountains in 2000 and 2013 under different temperature regimes. Shaded areas represent optimal temperature conditions for germination. The same letters are not significantly different ( $P > 0.05$ ) by the HSD Tukey test. Bars with dots and square symbol = 2000; Bars without dots and circle symbol = 2013; White = light; Black and grey = dark conditions.

**Table 2**  
Summary of the F-values of two-way ANOVA for the effects of temperature and years (2000 and 2013) and their interaction on germinability (G%) and germination rate (GR) of five species of bromeliads from the Serra da Piedade Mountains, southeastern Brazil.

Species	Light						Dark					
	Temperature		Years		Interaction		Temperature		Years		Interaction	
	G%	GR	G%	GR	G%	GR	G%	GR	G%	GR	G%	GR
<i>Vriesea friburgensis</i>	2.0 ns	6.1 ***	74.0 ***	309.9 ***	2.0 ns	2.6 *	2.6 ns	7.3 ***	90.8 ***	370.0 ***	3.0 *	4.2 **
<i>Vriesea bituminosa</i>	10.0 ***	148.4 ***	289.6 ***	377.9 ***	4.7 **	11.2 ***	11.0 ***	45.0 ***	107.4 ***	48.8 ***	2.0 ns	4.6 **
<i>Vriesea pardalina</i>	39.9 ***	111.9 ***	12.4 **	121.1 ***	22.7 *	39.4 ***	36.1 ***	45.6 ***	7.9 **	80.1 ***	18.4 ***	36.8 ***
<i>Tillandsia gardneri</i>	8.6 ***	158.6 **	137.2 ***	473.3 **	10.1 **	118.1 ***	0.05 ns	109.9 ***	58.9 ***	373.2 ***	1.3 ns	48.3 ***
<i>Racinaea aerisicola</i>	88.6 ***	11.9 ***	8.6 *	86.2 ***	19.9 ***	51.6 ***	39.3 ***	123.7 **	77.8 ***	780.4 **	1.7 ns	232.5 **

ns – not significant.

\* p < 0.05.

\*\* p < 0.01.

\*\*\* p < 0.001.

**Table 3**  
Base ( $T_b$ ) and ceiling ( $T_c$ ) temperatures and number of days ( $t_{50}$ ) until attaining a thermal-time for 50% seed germination [ $t_{50} = HS/(\Sigma(T_D - T_b))$ ] from the climatic data during the study period for each bromeliad species from the Serra da Piedade Mountains – MG. Two different IPCC scenarios (B2, +2.0 °C; and A2, +6.0 °C) were simulated.

Thermal Traits	<i>Vriesea friburgensis</i>		<i>Vriesea bituminosa</i>		<i>Vriesea pardalina</i>		<i>Tillandsia gardneri</i>		<i>Racinaea aerisicola</i>	
	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark
$T_{b2000}$ (°C)	6.5 ( $r^2 = 0.93$ )	6.2 ( $r^2 = 0.99$ )	8.5 ( $r^2 = 0.96$ )	8.0 ( $r^2 = 0.92$ )	8.5 ( $r^2 = 0.97$ )	8.5 ( $r^2 = 0.98$ )	7.5 ( $r^2 = 0.83$ )	7.5 ( $r^2 = 0.90$ )	10.0 ( $r^2 = 0.76$ )	10.0 ( $r^2 = 0.83$ )
$T_{b2013}$ (°C)	6.3 ( $r^2 = 0.92$ )	6.5 ( $r^2 = 0.95$ )	8.3 ( $r^2 = 0.95$ )	8.0 ( $r^2 = 0.94$ )	8.5 ( $r^2 = 0.94$ )	8.5 ( $r^2 = 0.95$ )	7.5 ( $r^2 = 0.90$ )	7.5 ( $r^2 = 0.84$ )	10.0 ( $r^2 = 0.97$ )	10.0 ( $r^2 = 0.96$ )
$T_{c2000}$ (°C)	39.3 ( $r^2 = 0.87$ )	39.4 ( $r^2 = 0.77$ )	41.3 ( $r^2 = 0.82$ )	41.6 ( $r^2 = 0.88$ )	39.7 ( $r^2 = 0.93$ )	40.6 ( $r^2 = 0.92$ )	40.8 ( $r^2 = 0.90$ )	40.8 ( $r^2 = 0.95$ )	36.8 ( $r^2 = 0.92$ )	36.8 ( $r^2 = 0.94$ )
$T_{c2013}$ (°C)	39.0 ( $r^2 = 0.87$ )	39.0 ( $r^2 = 0.86$ )	41.3 ( $r^2 = 0.94$ )	41.7 ( $r^2 = 0.91$ )	40.4 ( $r^2 = 0.91$ )	40.6 ( $r^2 = 0.94$ )	40.5 ( $r^2 = 0.97$ )	40.2 ( $r^2 = 0.91$ )	31.6 ( $r^2 = 0.92$ )	31.6 ( $r^2 = 0.84$ )
$t_{502000}$ (days)	1	1	4	11	11	14	2	4	3	4
$t_{502013}$ (days)	6	14	8	22	3	6	4	6	11	18
Climate prediction IPCC (B2–A2) ( $t_{50}$ –days), from:										
2000 data	1–1	1–1	3–4	8–10	7–9	8–11	1–1	3–3	2–3	3–3
2013 data	4–5	9–12	6–7	16–20	2–3	4–5	3–4	4–5	6–8	11–15

The  $D_i$  was calculated as follows:

$$D_i = P/PET, \quad (4)$$

where P is the precipitation and PET is the potential monthly evapotranspiration rate during each year.

Precipitation was higher (September–April, 292.2 mm more) with higher  $D_i$  and lower PET in 1999–2000 than in 2012–2013 (Fig. 3) during the periods of fruit ripening and seed development of *V. friburgensis*, *V. pardalina*, *V. bituminosa*, and *T. gardneri* (Fig. 2); *Racinaea aerisicola*, instead, developed and ripened its fruits and seeds during the dry period ( $D_i$  lower).

Slight differences in average temperatures ( $\sim 0.5$  °C) between the two study periods studied were observed in same season (Fig. 3). The minimum temperature was 14.8 °C and the maximum temperature was 30.3 °C in 1999–2000; and 15.3 and 30.4 °C in 2012–2013 respectively. The HS was calculated after seed dispersal at the end or beginning of the rainy period; from March (*V. bituminosa* and *T. gardneri*), April (*V. pardalina*), September (*V. friburgensis*), and October (*R. aerisicola*) (Fig. 2) as follows:

$$HS(\Sigma^{\circ}\text{Cd}) = \Sigma(\text{Env}T_D - T_b)t, \quad (5)$$

where  $T_b$  is the base temperature for germination,  $\text{Env}T_D$  is the average daily temperature during each period, and  $t$  is the number of days until attaining the median thermal-time value ( $\theta_{50}$ ).

IPCC scenarios of temperature increases by 2090–2099 relative to 1980–1999 were then simulated, adjusting the mean monthly temperatures according to the best and worst estimated tempera-

ture changes (+2 and +6 °C for the B2 and A2 scenarios respectively; IPCC, 2014). The number of days needed to reach the thermal-time for 50% seed germination ( $t_{50}$ ) was calculated from local average temperatures of the first days with rainfall after seed dispersal in the two study periods. The same calculations were performed to estimate the impacts of global temperature changes on germination time, adding the values 2 or 6 °C to the average local temperatures for each period.

### 3.3. Statistical analysis

The assumptions of the parametric analyses were tested through the Kolmogorov–Smirnov and Bartlett tests (Sileschi, 2012). Significant differences in germination parameters (G% and RG) were determined using two-way ANOVA, as it evaluates the effects of temperature and years (2000 and 2013) and the interactions between temperature and harvest years. Significant differences ( $P < 0.05$ ) between the treatments were evaluated using the multiple scale HSD Tukey test. Correlation analysis was performed using Pearson's correlation coefficient. All analyses were performed using the software Statistica 7.0.

## 4. Results

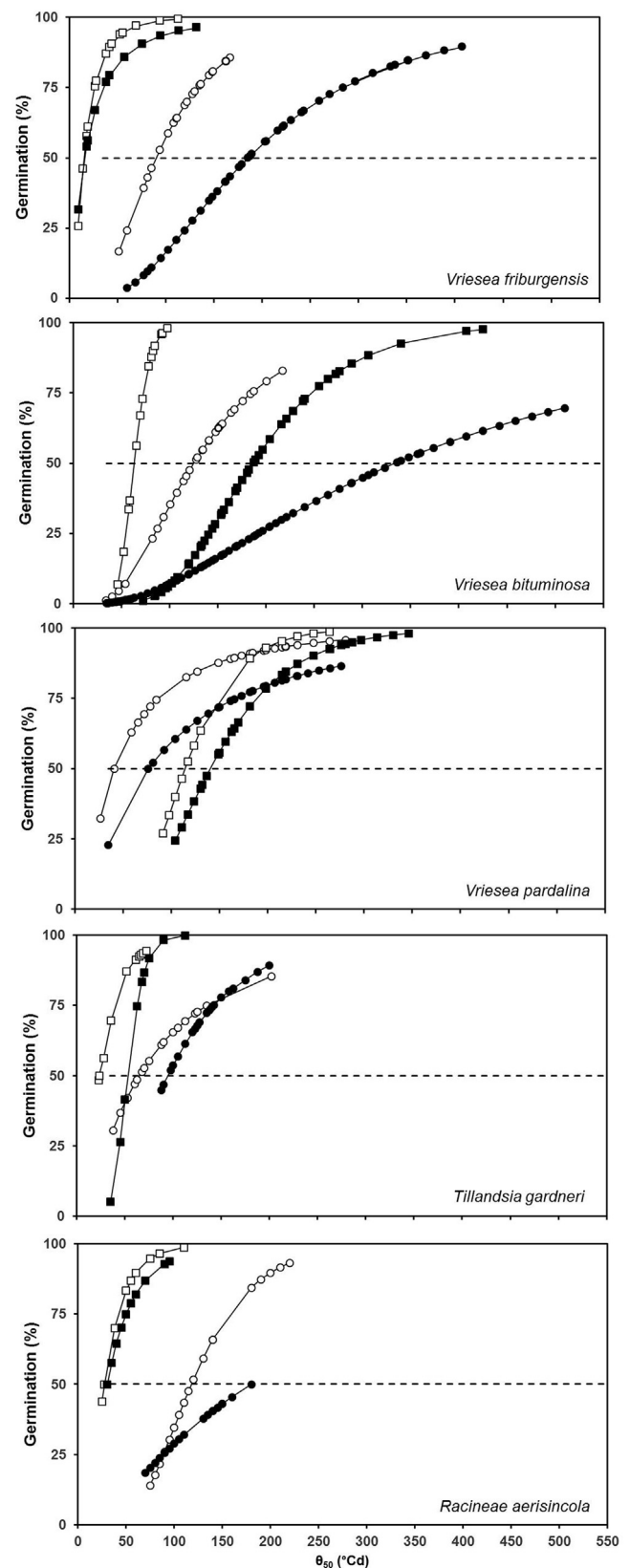
The optimal temperature conditions for germination varied between 25 and 30 °C for all species under both light and dark incubation, except for *R. aerisicola* (15 °C – dark; 20 °C – light) (Fig. 4).

In general, the G% under optimal conditions and GR decreased significantly ( $P < 0.001$ ) in all of the species that ripen their fruits and seeds during the period 2012–2013. Overall, the final germination percentage (G%) was higher (or equal) in light than in the dark (Fig. 4). The seeds of *V. friburgensis* germinated faster (GR=0.59 and 0.68 in the light and dark respectively) than to other species in 2000 and 2013 (Fig. 4); *R. aerisicola* had the lowest GR (0.05) for both years. GR had lower values overall under dark conditions. Both temperatures and years significantly affected the seed germination responses of the species under both light and dark conditions. Temperature had no effect on the germinability of *V. friburgensis* in 2000, as its germination values were nearly 100% across the entire temperature gradient under both light regimes (Fig. 4). GR was affected by temperatures and years in all five species under light and dark condition (Table 2). Temperatures by year interactions showed significant effects on the germinability of four species under light conditions and only two species under dark conditions; the effects were significant on the GR of all species under both light and dark conditions (Table 2).

The base temperatures for seed germination ( $T_b$ ) of the bromeliads populations for the two periods under light conditions ranged between 6.2 °C (*V. friburgensis*) to 10 °C (*R. aerisicola*) with few differences between seeds germinated on light and dark conditions (Table 3). The  $T_c$  values were approximately 40 °C for all populations (Table 3); except for *R. aerisicola* seeds, which demonstrated  $T_c$  equal to 36.8 °C for seeds harvested in 2000 and 31.6 °C for seeds from 2013. The breadth of the thermal niche (between  $T_b$  and  $T_c$ ) was larger in *Vriesea* species and *T. gardneri* (above 30 °C) and narrow in *R. aerisicola* (up to 26.8 °C) (Table 2).

Correlation analyses revealed significant associations ( $r > 0.9$ ) between germination progress curves obtained experimentally and the thermal time model fit obtained by probit analyses for the five bromeliads studied. The relationship between thermal time ( $\theta$ ) and G% for all species showed differences in thermal requirements between harvest years and experimental conditions (Fig. 5). The results showed that the  $\theta_{50}$  for seed populations of *V. friburgensis*, *V. bituminosa*, *T. gardneri* and *R. aerisicola* increased in 2013, while that of *V. pardalina* decreased (Fig. 5). *V. friburgensis*, *V. bituminosa* and *T. gardneri* populations experienced a more severe dry period (eight months with a drought index,  $D_i < 1.0$  year) during fruiting in 2013 (Fig. 2 and 3). The  $\theta_{50}$  for *V. bituminosa* and *T. gardneri* increased two-fold from one year to another (2000 → 2013) for seeds germinating in the light, and 1.8-fold under dark conditions. The thermal units needed to reach 50% germination were 11.7 times greater from 2000 → 2013 in dark conditions for *V. friburgensis* (15.7 → 184.3 °Cd). Fruiting and seed formation in *R. aerisicola* occurred only during the dry period (Fig. 3), with the  $\theta_{50}$  increasing more than 4-fold from 2000 → 2013. *V. pardalina* showed fruiting and seed formation only during the rainy season (summer), and its thermal requirements were lower in 2013 than in 2000 (Fig. 3); its  $\theta_{50}$  was two-fold lower under both light and dark conditions. The thermal requirements of all species were greater in the dark than under light condition (Fig. 5).

The  $t_{50}$  under different conditions (light, dark, year) varied from 2 to 6 days for *T. gardneri*, from 1 to 14 days for *V. friburgensis*, 3–14 days for *V. pardalina*, 3–18 days for *R. aerisicola*, and from 4 to 22 days for *V. bituminosa* (Table 3). The 2.0 °C increase predicted by the B2 scenario should lead to mean winter and summer temperatures (minimum and maximum) of approx. 16.6/21.1 °C and 28.1/30.7 °C respectively (from data for the year 2000) and 17.4/21.4 °C and 28.6/32.4 °C (from data for the year 2013). Additionally, an increasing heat sum would accelerate germination for all seeds populations (Table 3). A mean temperature increase of 6.0 °C (A2 scenario) should lead to mean winter and summer temperatures (minimum and maximum) of approx. 20.6/25.1 °C and 32.1/34.7 °C respectively (from data for the year 2000) and



**Fig. 5.** Seed germination (%) as a function of thermal time requirements ( $\theta$ , °Cd) for *Vriesea friburgensis*, *V. bituminosa*, *V. pardalina*, *Tillandsia gardneri*, and *Racineae aerisicola*. Thermal times were calculated assuming the base temperatures listed in Table 2. Thermal times to reach 50% of germination [ $\theta_{50}$ , corresponding to  $\text{probit}(g) = 5$ ] are shown for each seed lot by dashed lines. Square symbol = 2000; Circle symbol = 2013; White = light; Black = dark conditions.

21.4/25.4 °C and 32.6/36.4 °C respectively (from data for the year 2013). The increased heat sums by these predictions would further accelerate seed germination (Table 3).

## 5. Discussion

Germination rates in the presence and absence of light under optimum temperature condition were different among seeds harvested in different years. The bromeliad populations exhibited differences in their thermal times for germination in spite of the fact that there were no changes in the base ( $T_b$ ) and ceiling temperatures ( $T_c$ ). These results confirmed that  $T_b$  is constant within a population (Covell et al., 1986; Ellis et al., 1987; Yeh and Atherton, 2000; Hardgree, 2006; Andrade and Cardoso, 2016). The thermal thresholds identified for seed germination ( $T_b$  and  $T_c$ ) explained the differences in seed germination detected by Marques et al. (2014), and they are consistent with the idea that variations in germination responses to environmental factors are not random. The hypothesis that all sympatric bromeliads will show similar patterns of base ( $T_b$ ) and ceiling ( $T_c$ ) temperatures as well as thermal times was not, however, corroborated. Populations and genotypes that experience the greatest variability in a given environmental factor are expected to be the most plastic in terms of adaptive traits to those conditions (Van Tienderen 1991; Sultan and Spencer 2002; Valadares et al., 2014). Mota and Garcia (2013) observed that the tolerance of some *Vellozia* seeds to large thermal niches was consistent with the large daily temperature fluctuations they experienced in *campo rupestre* sites. In the face of anthropogenic changes, plasticity may play a key role in enabling persistence of remnant individuals or populations (Rubio de Casas et al., 2009; Chevin et al., 2010; Matesanz et al., 2010). Our study confirmed that habitat-generalist bromeliads (*V. bituminosa*, *V. friburgensis*, *V. pardalina* e *T. gardneri*) produce seeds that can germinate within larger thermal niches, whereas the habitat-specialist bromeliad (*R. aerisincola*) displayed a narrower thermal niche.

The inter-annual differences in thermal requirements of species may be explained by several factors, including their genetic origin (Coates and Byrne, 2005), seed position on the plant, and the age and growing environment of the parent during seed maturation (Wagner and Simons, 2009). In the present study, seeds from different harvest years were sampled in the same plant populations of each species, thus restricting genetic effects. Inter-annual differences in maternal environmental conditions represent the most likely explanation to account for the observed differences, as described in several other studies (Donohue et al., 2005; Luzuriaga et al., 2006; Kagaya et al., 2011).

Our results indicate that the extent and severity of the dry season during fruit ripening, linked to the microhabitats of bromeliads, seem to determine changes in the thermal requirements for seed germination. Sympatric bromeliad species with the same fruiting phenology (which include a period of drought) showed higher thermal requirements for seed germination in the driest year. These results corroborate our hypothesis that inter-annual differences in rainfall and temperature promote differences in the thermal requirements for germination. The effects of climate change, especially temperature, on plant regeneration niches have been studied extensively (Parmesan and Yohe, 2003; Bykova et al., 2012). However, the effects of drought during seed maturation on germination strategies are still only poorly known when considering bromeliad species. The conditions that enable germination have long-lasting consequences throughout the life of the plant (Donohue et al., 2010) because an appropriate germination response to environmental factors is the first requirement for successful establishment and growth. As such, germination timing can be a stringent selective filter, determining which genotypes became established under any

particular set of conditions (Donohue et al., 2005). For some species, the germination season determines the seasonal environments that a plant will experience throughout its life, since the sequence of seasons is, in general, quite predictable (Galloway, 2001, 2002).

It is important to consider the effects of environmental conditions during seed maturation on the maternal plant, in addition to the habitat of the bromeliad species. Marques et al. (2014) presented evidence that associations between seed germination traits and specific microhabitats provide examples of how habitat heterogeneity can modulate species coexistence at a local scale. Habitat heterogeneity may filter germination-related traits and thus play a determinant role in structuring heterogeneous plant communities. The authors suggested at least two germination strategies may have evolved in bromeliads to synchronize germination with optimum establishment conditions. First, germination synchrony increase with increasing soil moisture, and decrease with increasing environmental light; secondly, dark germination may reduce the probability of desiccation-induced death. Considering then the results of the present study, in addition to germination strategies that are related to the microhabitat where bromeliads occur, inter-annual differences in rainfall promote differences in the thermal parameters required for germination. Climate change will thus play a crucial role in controlling seed germination.

The maintenance of thermal thresholds, but with the plasticity of thermal requirements for germination being modulated by drought severity, may represent an important mechanism ensuring seedling establishment. In drier years the higher number of degree-days required for germination would prevent germination from occurring under episodes of sudden and rapid increases in humidity that are immediately followed by drought conditions. Differences among species in this plasticity will modulate their responses to the predicted climatic changes. If the predicted temperature rises occurs, the seeds of *Vriesea* species and *T. gardneri* should respond to decreases in the time required to sum heat units to germinate; *R. aerisincola* would likely suffer the most negative effects of temperature rises under both scenarios. In scenario A1, the mean minimum temperatures at the study site will be greater than 20 °C, which is above the  $T_o$  for seed germination in *R. aerisincola*. Cochrane et al. (2014) observed that populations that require lower temperatures for germination, or have narrower optimal ranges for germination, should be most vulnerable to a warming climate. Habitat-generalist bromeliads are likely to have greater chances of survival in face of climate change, whereas specialist species with narrow thermal range for germination are likely to go extinct, resulting in changes in the community on rocky outcrops in *campos rupestres*.

## Conflict of interest

None.

## Acknowledgements

The authors thank Dr. Victor José M. Cardoso for help with thermal-time model. We also wish to thank Dr. Fernando A. O. Silveira, who read the manuscript and offered many suggestions. This study was in partial fulfilment of the Ph.D requirements of A. A. Duarte at the Graduate Program in Plant Biology at the Universidade Federal de Minas Gerais, that received a fellowship from CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior). We acknowledge financial support by CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and FAPEMIG (Fundação de Amparo à Pesquisa do Estado de Minas Gerais).

## References

- Alves, R.J.V., Kolbek, J., 2010. Can campo rupestre vegetation be floristically delimited based on vascular plant genera? *Plant Ecol.* 207, 67–79, <http://dx.doi.org/10.1007/s11258-009-9654-8>.
- Andrade, L.F.D., Cardoso, V.J.M., 2016. Does thermal time for germination vary among populations of a tree legume (*Peltophorum dubium*)? *Braz. J. Biol.* 76, 592–599, <http://dx.doi.org/10.1590/1519-6984.18714>.
- Banta, J.A., Ehrenreich, I.M., Gerard, S., Chou, L., Wilczek, A., Schmitt, J., Kover, P.X., Purugganan, M.D., 2012. Climate envelope modelling reveals intraspecific relationships among flowering phenology, niche breadth and potential range size in *Arabidopsis thaliana*. *Ecol. Lett.* 15, 769–777, <http://dx.doi.org/10.1111/j.1461-0248.2012.01796>.
- Baskin, C.C., Baskin, J.M., 2014. *Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination*. Elsevier, San Diego.
- Benites, V.M., Schaefer, C.E.R., Simas, F.N.B., Santos, H.G., 2007. Soil associated with rock outcrops in the Brazilian mountain ranges Mantiqueira and Espinhaço. *Rev. Bras. Bot.* 30, 569–577, <http://dx.doi.org/10.1590/S0100-84042007000400003>.
- Bitencourt, C., Rapini, A., Damascena, L.S., Junior, P.M., 2016. The worrying future of the endemic flora of a tropical mountain range under climate change. *Flora* 218, 1–10, <http://dx.doi.org/10.1016/j.flora.2015.11.001>.
- Bradford, K.J., 1995. *Water relations in seed germination*. In: Kigel, J., Galili, G. (Eds.), *Seed Development and Germination*. Marcel Dekker, New York.
- Bykova, O., Chuine, I., Morin, X., Higgins, S.L., 2012. Temperature dependence of the reproduction niche and its relevance for plant species distributions. *J. Biogeogr.* 39, 2191–2200, <http://dx.doi.org/10.1111/j.1365-2699.2012.02764.x>.
- Chantre, G.R., Batlla, D., Sabbatini, M.R., Orioli, G., 2009. Germination parameterization and development of an after-ripening thermal-time model for primary dormancy release of *Lithospermum arvense* seeds. *Ann. Bot. London* 103, 1291–1301.
- Chevin, L.-M., Lande, R., Mace, G.M., 2010. Adaptation, plasticity, and extinction in a changing environment: towards a predictive theory. *PLoS Biol.* 8, e1000357, <http://dx.doi.org/10.1371/journal.pbio.1000357>.
- Coates, D., Byrne, M., 2005. Genetic variation in plant populations: assessing cause and pattern. In: Henry, R. (Ed.), *Plant Diversity and Evolution: Genotypic and Phenotypic Variation in Higher Plants*. CABI Publishing, Cambridge.
- Cochrane, A., Hoyle, G.L., Yates, C.J., Wood, J., Nicotra, A.B., 2014. Predicting the impact of increasing temperatures on seed germination among populations of Western Australian *Banksia* (Proteaceae). *Seed Sci. Res.* 24, 195–205, <http://dx.doi.org/10.1017/S096025851400018X>.
- Conceição, A.A., Pirani, J.R., 2016. Succession on the rocky outcrop vegetation: a rupestrian grassland scheme. In: Fernandes, G.W. (Ed.), *Ecology and Conservation of Mountaintop Grasslands in Brazil*, Chapter 9. Springer International Publishing, New York, pp. 181–206, <http://dx.doi.org/10.1007/978-3-319-29808-5>.
- Covell, S., Ellis, R.H., Roberts, E.H., Summerfield, R.J., 1986. The influence of temperature on seed germination rate in grain legumes. I. A comparison of chickpea lentil, soyabean, and cowpea at constant temperatures. *J. Exp. Bot.* 37, 705–715.
- Daws, M.I., Lydall, E., Chmielarz, P., Leprince, O., Matthews, S., Thanos, C.A., Pritchard, H.W., 2004. Developmental heat sum influences recalcitrant seed traits in *Aesculus hippocastanum* across Europe. *New Phytol.* 162, 157–166, <http://dx.doi.org/10.1111/j.1469-8137.2004.01012.x>.
- Donohue, K., Dorn, L., Griffith, C., Kim, E., Aguilera, A., Polisetty, C.R., Schmitt, J., 2005. Environmental and genetic influences on the germination of *Arabidopsis thaliana* in the field. *Evolution* 59, 740–757, <http://dx.doi.org/10.1111/j.0014-3820.2005.tb01750.x>.
- Donohue, K., Casas, R.R., Burghardt, L., Kovach, K., Willis, C.G., 2010. Germination, postgermination adaptation, and species ecological ranges. *Annu. Rev. Ecol. Syst.* 41, 293–319, <http://dx.doi.org/10.1146/annurev-ecolsys-102209-144715>.
- Ellis, R.H., Covell, S., Roberts, E.H., Summerfield, R.J., 1986. The influence of temperature on seed germination rate in grain legumes II. Intraspecific variation in chickpea (*Cicer arietinum* L.) at constant temperatures. *J. Exp. Bot.* 37, 1503–1515.
- Fernandes, G.W., 2016. *Ecology and Conservation of Mountaintop Grasslands in Brazil*. Springer International Publishing, New York, <http://dx.doi.org/10.1007/978-3-319-29808-5>.
- Finney, D.J., 1971. *Probit Analysis*. Cambridge University Press, Cambridge.
- Galloway, L.F., 2001. Parental environmental effects on life history in the herbaceous plant *Campanula americana*. *Ecology* 82, 2781–2789, [http://dx.doi.org/10.1890/0012-9658\(2001\)08\[2781:PEEOLH\]2.0.CO;2](http://dx.doi.org/10.1890/0012-9658(2001)08[2781:PEEOLH]2.0.CO;2).
- Galloway, L.F., 2002. The effect of maternal phenology on offspring characters in the herbaceous plant *Campanula americana*. *J. Ecol.* 90, 851–858, <http://dx.doi.org/10.1046/j.1365-2745.2002.00714.x>.
- García-Huidobro, J., Monteith, J.L., Squire, G.R., 1982. Time, temperature and germination of pearl millet (*Pennisetum typhoides* S. & H.) I. constant temperature. *J. Exp. Bot.* 33, 288–296, <http://dx.doi.org/10.1111/j.1365-2486.2009.01851.x>.
- Givnish, T.J., Barfuss, M.H.J., Van Ee, B., Riina, R., Schulte, K., Horres, R., Gonsiska, P.A., Jabaily, R.S., Crayn, D.M., Smith, J.A.C., Winter, K., Brown, G.K., Evans, T.M., Holst, B.K., Luther, H., Till, W., Zizka, G., Berry, P.E., Sytsma, K.J., 2011. Phylogeny, adaptive radiation, and historical biogeography in Bromeliaceae: insights from an eight-locus plastid phylogeny. *Am. J. Bot.* 98, 872–895, <http://dx.doi.org/10.3732/ajb.1000059>.
- Gordo, O., Sanz, J.J., 2009. Long-term temporal changes of plant phenology in the Western Mediterranean. *Glob. Change Biol.* 15, 1930–1948, <http://dx.doi.org/10.1111/j.1365-2486.2009.01851.x>.
- Gordo, O., Sanz, J.J., 2010. Impact of climate change on plant phenology in Mediterranean ecosystems. *Glob. Change Biol.* 16, 1082–1106, <http://dx.doi.org/10.1111/j.1365-2486.2009.02084.x>.
- Govaerts, R., Luther, H.E., Grant, J., 2012. World Checklist of Bromeliaceae. Kew: Royal Botanic Gardens (Available at: <http://apps.kew.org/wcsp/> (retrieved 29, May 2016)).
- Gratani, L., 2014. Plant phenotypic plasticity in response to environmental factors. *Adv. Bot.* 17, <http://dx.doi.org/10.1155/2014/208747> (article ID 208747).
- Hardegee, S.P., 2006. Predicting germination response to temperature. I. Cardinal-temperature models and subpopulation-specific regression. *Ann. Bot.* 97, 1115–1125, <http://dx.doi.org/10.1093/aob/mcl163>.
- Hay, F.R., Mead, A., Bloomberg, M., 2014. Modelling seed germination in response to continuous variables: use and limitations of probit analysis and alternative approaches. *Seed Sci. Res.* 24, 165–186, <http://dx.doi.org/10.1017/S096025851400021X>.
- Hoffmann, A.A., Camac, J.S., Williams, R.J., Papst, W., Jarrad, F.C., Wahren, C.H., 2010. Phenological changes in six Australian subalpine plants in response to experimental warming and year-to-year variation. *J. Ecol.* 98, 927–937, <http://dx.doi.org/10.1111/j.1365-2745.2010.01667.x>.
- IPCC, 2014. *Climate Change 2014 Synthesis Report. An Assessment of the Intergovernmental Panel on Climate Change*. IPCC, Geneva.
- IUCN, 2001. *IUCN Red List Categories and Criteria: Version 3.1 IUCN Species Survival Commission*. IUCN, Gland Switzerland, Cambridge.
- Jacobi, C.M., Carmo, F.F., Vicent, R.C., Stehmann, J.R., 2007. Plant communities on ironstone outcrops: a diverse and endangered Brazilian ecosystem. *Biodivers. Conserv.* 16, 2185–2200, <http://dx.doi.org/10.1007/s10531-007-9156-8>.
- Kagaya, M., Tani, T., Kachi, N., 2011. Maternal and paternal effects on the germination time of non-dormant seeds of a monocarpic perennial species, *Aster kantoensis* (Compositae). *Plant Species Biol.* 26, 66–72, <http://dx.doi.org/10.1111/j.1442-1984.2010.00303.x>.
- Luther, H.E., 2008. *An Alphabetical List of Bromeliad Binomials*. The Bromeliad Society International Inc., Orlando-Florida.
- Luzuriaga, A.L., Escudero, A., Pérez-García, F., 2006. Environmental effects on seed morphology and germination in *Sinapis arvensis* (Cruciferae). *Weed Res.* 46, 163–174, <http://dx.doi.org/10.1111/j.1365-3180.2006.00496.x>.
- Müller, L.L.B., Albach, D.C., Zotz, G., 2016. 'Are 3 °C too much?': thermal niche breadth in Bromeliaceae and global warming. *J. Ecol.* <http://dx.doi.org/10.1111/1365-2745.12681>.
- Marques, A.R., Lemos-Filho, J.P., 2008. Reproductive phenology of bromeliad species at Serra da Piedade, Minas Gerais state, Brazil. *Acta Bot. Bras.* 22, 417–424, <http://dx.doi.org/10.1590/S0102-33062008000200011>.
- Marques, A.R., Lemos-Filho, J.P., Mota, R.C., 2012. Diversity and conservation status of bromeliads from Serra da Piedade, Minas Gerais, Brazil. *Rodriguésia* 63, 243–255, <http://dx.doi.org/10.1590/S2175-78602012000200001>.
- Marques, A.R., Atman, A.P.F., Silveira, F.A.O., Lemos-Filho, J.P., 2014. Are seed germination and ecological breadth associated? Testing the regeneration niche hypothesis with bromeliads in a heterogeneous neotropical montane vegetation. *Plant Ecol.* 215, 517–529, <http://dx.doi.org/10.1007/s11258-014-0320-4>.
- Matesanz, S., Gianoli, E., Valladares, F., 2010. Global change and the evolution of phenotypic plasticity in plants. In: Schlichting, C.D., Mousseau, T.A. (Eds.), *Year in Evolutionary Biology*. Wiley-Blackwell, Malden, pp. 35–55.
- McNair, J.N., Sunkara, A., Frobish, D., 2012. How to analyse seed germination data using statistical time-to-event analysis: non-parametric and semi-parametric methods. *Seed Sci. Res.* 22, 77–95, <http://dx.doi.org/10.1017/S0960258511000547>.
- Meineri, E., Spindelböck, J., Vandvik, V., 2013. Seedling emergence responds to both seed source and recruitment site climates: a climate change experiment combining transplant and gradient approaches. *Plant Ecol.* 214, 607–619, <http://dx.doi.org/10.1007/s11258-013-0193-y>.
- Menzel, A., Sparks, T.H., Estrella, N., Koch, E., Aasa, A., Ahas, R., Alm-kubler, K., Bissolli, P., Braslavská, O., Briede, A., Chmielewski, F.M., Crepinsek, Z., Curnel, Y., Dahl, A., Defila, C., Donnelly, A., Filella, Y., Jactzak, K., Mage, F., Mestres, A., Nordli, Ø., Peñuelas, J., Pirinen, P., Remisová, V., Scheffinger, H., Striz, M., Susnik, A., Van Vliet, A.J.H., Wielgolaski, F.-E., Zach, S., Züst, A., 2006. European phenological response to climate change matches the warming pattern. *Glob. Change Biol.* 12, 1969–1976, <http://dx.doi.org/10.1111/j.1365-2486.2006.01193.x>.
- Molina-Montenegro, M.A., Atala, C., Gianoli, E., 2010. Phenotypic plasticity and performance of *Taraxacum officinale* (dandelion) in habitats of contrasting environmental heterogeneity. *Biol. Invasions* 12, 2277–2284.
- Moreira, A.S.F.P., Borba, E.L., Lemos-Filho, J.P., 2013. Testing arbitrary classes of light in a physiognomically heterogeneous area of campo rupestre vegetation. *Ann. Acad. Bras. Cienc.* 85, 635–648, <http://dx.doi.org/10.1590/S0001-37652013005000026>.
- Mota, L.A.S., Garcia, Q.S., 2013. Germination patterns and ecological characteristics of *Vellozia* seeds from high-altitude sites in south-eastern Brazil. *Seed Sci. Res.* 23, 67–74, <http://dx.doi.org/10.1017/S0960258512000256>.
- Nicotra, A.B., Atkin, O.K., Bonser, S.P., Davidson, A.M., Finnegan, E.J., Mathiesius, U., Richards, P.C.L., Valladares, F., van Kleunen, M., 2010. Plant phenotypic plasticity in a changing climate. *Trends Plant Sci.* 15, 684–692, <http://dx.doi.org/10.1016/j.tplants.2010.09.008>.

- Pérez-García, F., 2009. Germination characteristics and intrapopulation variation in carob (*Ceratonia siliqua* L.) seeds. *Span. J. Agric. Res.* 7, 398–406, <http://dx.doi.org/10.5424/sjar/2009072-431>.
- Parmesan, C., Yohe, G., 2003. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* 421, 37–42, <http://dx.doi.org/10.1038/nature01286>.
- Picotte, J.J., Rhode, J.M., Cruzan, M.B., 2009. Leaf morphological responses to variation in water availability for plants in the *Piriqueta caroliniana* complex. *Plant Ecol.* 200, 267–275, <http://dx.doi.org/10.1007/s11258-008-9451-9>.
- Porceddu, M., Mattana, E., Pritchard, H.W., Bacchetta, G., 2013. Thermal niche for in situ seed germination by Mediterranean mountain streams: model prediction and validation for *Rhamnus persicifolia* seeds. *Ann. Bot.* 112, 1887–1897, <http://dx.doi.org/10.1093/aob/mct238>.
- Post, E.S., Pedersen, C., Wilmers, C.C., Forchhammer, M.C., 2008. Phenological sequences reveal aggregate life history response to climatic warming. *Ecology* 89, 363–370, <http://dx.doi.org/10.1890/06-2138.1>.
- Ranal, M.A., Santana, D.G., 2006. How and why to measure the germination process? *Bras. Bot.* 29, 1–11, <http://dx.doi.org/10.1590/S0100-84042006000100002>.
- Rubio de Casas, R., Vargas, P., Perez-Corona, E., Cano, E., Manrique, E., Garcia-Verdugo, C., Balaguer, L., 2009. Variation in sclerophylly among Iberian populations of *Quercus coccifera* L. is associated with genetic differentiation across contrasting environments. *Plant Biol.* 11, 464–472, <http://dx.doi.org/10.1111/j.1438-8677.2008.00128.x>.
- Sales, N.M., Pérez-García, F., Silveira, F.A.O., 2013. Consistent variation in seed germination across an environmental gradient in a Neotropical savanna. *S. Afr. J. Bot.* 87, 129–133, <http://dx.doi.org/10.1016/j.sajb.2013.04.001>.
- Schaefer, C.E., Corrêa, G.R., Candido, H.G., Arruda, D.M., Nunes, J.A., Araujo, R.W., Rodrigues, P.M.S., Filho, E.I.F., Pereira, A.F.S., Brandão, P.C., Neri, A.V., 2016. The physical environment of rupestrian grasslands (Campos Rupestres) in Brazil: geological, geomorphological and pedological characteristics, and interplays. In: Fernandes, G.W. (Ed.), *Ecology and Conservation of Mountaintop Grasslands in Brazil*, Chapter 2. Springer International Publishing, New York, pp. 15–53, <http://dx.doi.org/10.1007/978-3-319-29808-5>.
- Sileshi, G.W., 2012. A critique of current trends in the statistical analysis of seed germination and viability data. *Seed Sci. Res.* 22, 145–159, <http://dx.doi.org/10.1017/S0960258512000025>.
- Silveira, F.A.O., Negreiros, D., Barbosa, N.P., Buisson, E., Carmo, F.F., Carstensen, D., Conceição, A.A., Cornelissen, T.G., Echternacht, L., Fernandes, G.W., Garcia, Q.S., Guerra, T.J., Jacobi, C.M., Lemos-Filho, J.P., Schaefer, C.E., Viana, P., Lambers, H., 2016. Ecology and evolution of plant diversity in the endangered campo rupestre: a neglected conservation priority. *Plant Soil* 403, 129–152, <http://dx.doi.org/10.1007/s11104-015-2637-8>.
- Sultan, S.E., Spencer, H.G., 2002. Metapopulation structure favors plasticity over local adaptation. *Am. Nat.* 160, 271–283, <http://dx.doi.org/10.1086/341015>.
- Valladares, F., Gianoli, E., Gomez, J.M., 2007. Ecological limits to plant phenotypic plasticity. *New Phytol.* 176, 749–763, <http://dx.doi.org/10.1111/j.1469-8137.2007.02275.x>.
- Valladares, F., Matesanz, S., Guilhaumon, F., Araújo, M.B., Balaguer, L., Benito-Garzón, M., Cornwell, W., Gianoli, E., Kleunem, M.V., Naya, D.E., Nicotra, A.B., Poorter, H., Zavala, M.A., 2014. The effects of phenotypic plasticity and local adaptation on forecasts of species range shifts under climate change. *Ecol. Lett.* 17, 1351–1364, <http://dx.doi.org/10.1111/ele.12348>.
- Van Tienderen, P.H., 1991. Evolution of generalists and specialist in spatially heterogeneous environments. *Evolution* 45, 1317–1331.
- Versieux, L.M., Wendt, T., 2007. Bromeliaceae diversity and conservation in Minas Gerais state, Brazil. *Biodivers. Conserv.* 16, 2989–3009, <http://dx.doi.org/10.1007/s10531-007-9157-7>.
- Versieux, L.M., Louzada, R.B., Viana, P.L., Mota, N., Wanderley, M.G.L., 2010. An illustrated checklist of Bromeliaceae from Parque Estadual do Rio Preto Minas Gerais, Brazil, with notes on phytogeography and one new species of *Cryptanthus*. *Phytotaxa* 10, 1–16.
- Vicente-Serrano, S.M., Beguería, S., López-Moreno, J.I., 2010. A multiscalar drought index sensitive to global warming: the standardized precipitation evapotranspiration index. *J. Clim.* 23, 1696–1718, <http://dx.doi.org/10.1175/2009JCLI2909.1>.
- Wagner, I., Simons, A.M., 2009. Divergence among arctic and alpine populations of the annual, *Koenigia islandica*: morphology, life-history, and phenology. *Ecography* 32, 114–122, <http://dx.doi.org/10.1111/j.1600-0587.2008.05497.x>.
- Yeh, D.M., Atherton, J.G., 2000. Cardinal temperatures and thermal requirements for germination of cineraria seed. *J. Hort. Sci. Biotechnol.* 75, 476–480, <http://dx.doi.org/10.1080/14620316.2000.11511272>.